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STUDIES IN THE RHODOPHYLLOID FUNGI. I. GENERIC CONCEPTS

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This paper is concerned with the Rhodophyllaceae in the sense of Singer (except *Clitopilus* and *Rhodocybe*).

In 1821 Elias Fries classified the rhodophylloid fungi into tribes which were distinguished by variations of the following features: 1, consistency of the carpophore, particularly of the stipe, 2, attachment of the lamellae, 3, shape of the pileus, and 4, nature of the pileal surface. In 1838 he further emphasized the nature of the pileal surface as a diagnostic feature, using it as well as other characteristics to divide the tribe *Entoloma* into three sections, and admitted species with flocculose pilei to tribe *Nolanea*, which previously had contained only mushrooms with glabrous pilei. In subsequent publications Fries no longer used the pileal surface in diagnostic characterizations but defined his tribes (which he now called subgenera) only on the basis of consistency of stipe, type of pileal margin, and attachment of lamellae. These three features have continued to be used by mycologists who have chosen to maintain the Friesian groupings, whether at the generic or subgeneric level. Unfortunately all three are variable, or hard to assess, or both. The difficulty of accurately defining taxa by such features has led some

contemporary mycologists to place all rhodophylloid fungi in a single genus, *Rhodophyllus* Quel., or *Entoloma* (Fr.) Kummer emend. Donk, characterized by pink, angular spores, subparallel lamellar trama, and attached lamellae.

This solution does indeed create a clearly defined genus for the rhodophylloid fungi, but it avoids the questions of what characteristics would be more satisfactory than those used by Fries to delineate subgeneric taxa. In recent years some efforts have been made to find such characters. Romagnesi (in Kühner and Romagnesi, 1953) and Hesler (1967) have emphasized microscopic characters more than macroscopic ones. Smith and Shaffer (1964) have suggested using diameter rather than consistency of the stipe; they define a fleshy-fibrous stipe as one with a diameter at the apex greater than 5 mm, and a cartilaginous stipe as one with the apex less than 5 mm broad. The probable value of urea as a chemotaxonomic aid in separating certain groups of the rhodophylloid fungi was shown by Tyler, et al. (1965).

The present study proposes the use of the anatomy and the general aspect of the pileal surface as important diagnostic characters, and correlates concentration of urea with various microscopic and macroscopic features.

AFFINITIES AND CHARACTERIZATION

The rhodophylloid fungi have pink to vinaceous spores that are angular in face, side, and end views, lamellar trama composed of subparallel hyphae, and lamellae that are variously attached but never distinctly free. These fungi are closely related to species of *Clitopilus* and of *Rhodocybe* which have spores that are angular in end view. In *Clitopilus*, the spores are longitudinally grooved or striate when seen in side or face view; in *Rhodocybe*, the spores are roughened to warty when seen in face or side view. Several other genera also have pink spores, but differ from the rhodophylloid genera in other ways. For example, the punctate-roughened spores of *Lepista* are not angular in any view. *Rhodotus* has smooth to echinulate spores, free lamellae, and divergent lamellar trama. *Volvariella*, *Chamaecota*, and *Pluteus* all possess smooth spores, convergent lamellar trama, and free gills; furthermore, *Volvariella* has a volva, *Chamaecota* an annulus.

CHROMATOGRAPHIC METHODS

One hundred mg of finely ground gill, pileus and stipe tissue was placed in a screw cap vial, along with 1.5 ml ethanol and agitated vigorously on a rotary shaking machine for approximately 24 hours. Fifty μ l of clear supernatant of each extract was spotted one inch from the base of $9\frac{1}{4} \times 22\frac{1}{2}$ ", oxalic-acid-washed Whatman No. 1 filter paper, together with reference spots of 5, 10 and 25 μ g of urea respectively. The chromatogram was developed with a wash liquid composed of n-butanol-acetic acid-water (4:1:1) for approximately 18 hours. Four to five chromatograms were run simultaneously in each chromatographic

TABLE 1. UREA AS A CHEMOTAXONOMIC INDICATOR IN CERTAIN RHODOPHYLLOID FUNGI

Genera (sensu Dennis, <i>et al.</i>)	Number of Species	Urea Concentrations
<i>Claudopus</i>	1 (2)*	5
<i>Nolanea</i>	5 (34)	5
<i>Entoloma</i>	7 (17)	0 - 2
<i>Leptonia</i>	5 (42)	0 - 0.3

*Figures in parentheses are number of collections.

chamber located in a constant temperature room at 25°C. The chromatograms were dried at room temperature, marked to show the location of fluorescent spots and then sprayed with 2% p-dimethylaminobenzaldehyde in hydrochloric acid (Ehrlich's reagent, hereinafter referred to as PDAB). (Ehrlich's reagent: p-dimethylaminobenzaldehyde (PDAB), 6 grams; Ethanol, 95%, 229 ml; and Concentrated hydrochloric acid, 71 ml. If the spray is stored in a colored bottle and kept below room temperature, it should remain stable for extended periods.)

The Rf values were calculated for all unknown compounds and for three urea standards. The sizes and color intensities of yellow spots with the same mobility as the three standards were compared with the latter. Extracts rated 0-1 contained none to approximately 5 µg/spot; 2, around 10 µg; 4, about 25 µg and 5, more than 25 µg.

RESULTS

Although 77 compounds were detected in the 279 extracts examined, only those compounds forming a yellow chromophore at the same Rf value as those of the urea standards (0.50 ± 0.06) are of chemotaxonomic concern in the present study. Ninety-five of the 279 collections analyzed in the above manner were identified to species by classical microscopic and macroscopic techniques. The placement of these species into genera (sensu Dennis, *et al.*, 1960), together with data on their urea concentrations, are shown in Table 1.

The data in Table 2 include *Pouzaromyces* Pilát and a segregate of *Leptonia* (*L. sericella* species complex). The data further show that urea levels in all collections placed in *Nolanea* were consistently high, whereas those in *Leptonia* and *Entoloma* were much lower.

TABLE 2. UREA IN 279 COLLECTIONS OF RHODOPHYLLOID FUNGI

Genera		Urea Concentrations			
		0 - 1	2	4	5
<i>Leptonia sericella</i>	15*	15	0	0	0
species complex					
<i>Pouzaromyces</i>	2	0	1	0	1
<i>Leptonia</i>	86	63	15	3	5
<i>Entoloma</i>	66	40	23	2	1
<i>Nolanea</i>	108	0	0	1	107
<i>Claudopus</i>	2	0	0	0	2
Total	279				

*Number of collections examined.

KEY TO THE GENERA

- Stipe eccentric, lateral, or lacking *Claudopus*
 Stipe central.
 Pileal surface in radial section a layer of repent, radially oriented hyphae.
 Urea concentration low (0 to +1, rarely +2); clamps usually present on 20% or more of the pileal cuticular hyphae; carpophores large and fleshy (pileal trama 3 mm or more at edge of umbo, apex of stipe 5 mm or more in diam. *Entoloma*
 Urea concentration high to very high (+4 to +5); clamps absent or if present, on less than 10% of the pileal cuticular hyphae; carpophores usually small or medium sized, and thin-fleshed (pileal trama 2 mm or less, apex of stipe less than 5 mm), but sometimes large and fleshy as in *Entoloma* . *Nolanea*
 Pileal surface in radial section an entangled to irregularly interwoven trichodermium, a palisade trichodermium, an ixotrichodermium, or a hymeniform layer (at least when young).
 Urea concentration high to very high (+4 to +5); pileus thin-fleshed, conic to campanulate, often with a papillate or cuspidate umbo, the cuticle often overlain by a colorless superficial layer or veil *Nolanea*
 Urea concentration low to medium (0 to +3); pileus varying in shape and thickness of flesh, but not conical with papillate or cuspidate umbo.
 Pileal cuticle a palisade trichodermium, or hymeniform, at least on the disc *Leptonia*
 Pileal cuticle an entangled to an irregularly interwoven trichodermium or an ixotrichodermium.
 Terminal cells of pileal cuticle averaging less than 8 μ wide . . . *Entoloma*
 Terminal cells averaging 8 μ or more wide.
 Young, fresh carpophores entirely white to pale cinereous. *Alboleptonia*
 Carpophores not entirely white to pale cinereous.
 Cuticle at apex of stipe similar to pileal cuticle.
 Cuticular hyphae of the pileal disc 190-600 μ long, 5-10(-15)-septate, average length greater than 250 μ *Pouzaromyces*
 Cuticular hyphae of the pileal disc up to but not exceeding 300 μ in length, (1-)3-5-septate, average length 225 μ or less . *Leptonia*
 Stipe and pileal cuticles not similar.
 Caulocystidia, if present, versiform but not indense clusters . *Leptonia*
 Caulocystidia obclavate to aculeate and in rosette-like clusters.
Pouzaromyces

GENERA

CLAUDOPUS (W. G. Smith) Gill., emend Pat, Les Hyménomycètes d'Europe, 113. 1887. Basionym: *Agaricus* subgenus *Claudopus* W. G. Smith, Clavis Agaricinorum. Type species: *Agaricus byssisedus* Pers. *per* Fr.

In members of this genus the carpophore often lacks a stipe; when one is present, it is always lateral or eccentric. Also, the habit of the carpophores of growing on the underside of logs or of shelving clumps of moss has not been encountered in other fungi with pink, angular spores. *Leptonia dichroa* and related lignicolous species have a tricholomatoid carpophore.

Extracts from only two collections of *Claudopus* were chromatographed. Each possessed a high concentration of urea, but otherwise had no distinctive Erlich-positive spots.

ENTOLOMA (Fr.) Kummer, *Der Führer in die Pilzkunde*, 23, 97. 1871. emend. Basionym: *Agaricus* tribus *Entoloma* Fr., *Epicrisis*, 143. 1838. Synonym: *Agaricus* subgenus *Entoloma* (Fr.) Rabenh., *Deutschl. Krypt.-Fl.* 1:508. 1844. Type species: *Agaricus prunuloides* Fr.

Pileus: glabrous, at times with a pruina of whitish or grayish fibrils, or with minute colorless squamules; surface dry, lubricous, or viscid; context 3 mm or more thick at the edge of the umbo. Stipe: 5 mm or more broad at the apex. Pileal cuticle: in radial section, either of repent hyphae, or an entangled to interwoven trichodermium, or an ixotrichodermium; terminal cells long, thin, claviform, averaging less than 8.0 μ wide; hypoderm similar to the pileal cuticle. Clamp connections: on at least 20% or more of the septa of all pileal cuticular hyphae, long, thin, at times rare. Pigmentation: vacuolar, rarely externally incrustated. Urea concentration: low (0 to +1).

Deviations from one or another of these characteristics are encountered. For instance, some specimens have very few clamps on the pileal cuticular hyphae, but are recognizable as *Entolomas* by their fleshy, tricholomatoid carpophores with glabrous pileus and low urea content. In other specimens the pileus instead of being glabrous is covered with a pruina of whitish or grayish fibrils, or with minute colorless squamules, and in still others, externally incrusting pigment replaces vacuolar pigmentation. But in all of these instances, however, the specimen can be identified as an *Entoloma* by its fleshy carpophore, low urea content, and relatively abundant clamp connections.

NOLANEA (Fr.) Kummer, *Der Führer in die Pilzkunde*, 24, 95. 1871. emend. Basionym: *Agaricus* tribus *Nolanea* Fr., *Syst. Mycol.* 1:204. 1821. Type species: *Agaricus pascuus* Fr.

Pileus: convex to campanulate or conic, rarely depressed or umbilicate, often umbonate, papillate, or cuspidate; surface glabrous, at times overlaid with a superficial veil, dry or lubricous; context 2(-5) mm or less thick at the center or at the edge of the umbo. Stipe: 4(-8) mm or less broad at the apex. Pileal cuticle: in radial section either of repent hyphae or an entangled to interwoven trichodermium; hypoderm often differentiated. Clamp connections: absent or if present, on less than 10% of the septa of all pileal cuticular hyphae, rarely more abundant. Pigmentation: vacuolar or externally incrustated. Urea Concentration: high (+4 to +5).

In occasional specimens the pileus is depressed to umbilicate, but more often, the carpophore is almost tricholomatoid in stature, and has clamps on more than 10% of the septa in the pileal surface. In both instances the fungus can be included in *Nolanea*, because the specimens also possess the distinctive combination of high urea content and repent pileal cuticle.

Nolanea can be distinguished from *Entoloma* by its pileal cuticle whose hyphae are well differentiated from those of the pileal trama, by the absence or rarity of clamp connections, the less fleshy carpophore,

and more reliably by the high urea concentration. *Leptonia*, *Pouzaromyces*, and the *Leptonia sericella* species complex all differ from *Nolanea* by virtue of the different structure of their pileal cuticle, and their low urea content.

POUZAROMYCES Pilát, Sborn. Národn. Mus. Praze 9B (2):60. 1953.
Type species: *Agaricus fumosellus* Winter.

The species of this genus are distinguished by their dark brown to gray-brown tomentulose to densely scaly pilei, initially dark brown to gray-brown lamellae, and pruinose to densely scaly stipes that are concolorous with the pilei. Cheilocystidia are present on the lamellar edge. The pileal cuticle is composed of septate, entangled hyphae with dark brown vacuolar pigment and some externally incrustated pigment. The cuticle at the apex of the stipe is either similar to the pileal cuticle, or bears rosette-like clusters of long, obclavate to aculeate caulocystidia.

Pouzaromyces can be distinguished from *Entoloma*, *Nolanea*, the *Leptonia sericella* species complex, and most other *Leptonias* on the basis of color, and of the nature of the surface and cuticle of both pileus and stipe. Certain species of *Leptonia* (e.g., *L. subdysthales*) resemble *Pouzaromyces* in general appearance, especially as to color, but differ in the structure of the pileal cuticle, or the cuticle of the stipe, or both.

LEPTONIA (Fr.) Kummer, Der Führer in die Pilzkunde, 24, 96. 1871. emend. Basionym: *Agaricus* tribus *Leptonia* Fr., Syst. Mycol. 1:201. 1821. Synonyms: *Eccilia* (Fr.) Kummer, Der Führer in die Pilzkunde, 23, 94. 1871. *Leptoniella* Earle, Bull. N. Y. Botan. Gard. 5:424. 1909. Type of species: *Agaricus euchrous* Pers. *per* Fries.

Pileus: tomentulose or punctate or squamose to squamulose, at least on the disc, at times silky-, matted-, or appressed-fibrillose. Pileal cuticle (in radial section and at least on the disc): an entangled trichodermium, hymeniform, or a palisade trichodermium; pilocystidia or cystidioid terminal cells 8μ or more in diameter. Clamp connections: present or absent. Pigmentation: vacuolar or rarely incrustated. Urea concentration: variable (0 to +3).

The species of *Leptonia* are characterized primarily by the nature of the pileal surface and the structure of the pileal cuticle. The surface is tomentulose or punctate or squamose to squamulose, at least on the disc, and the cuticle is an entangled trichodermium of agglutinated or non-agglutinated hyphae, or is hymeniform, or a palisade trichodermium. Pilocystidia or cystidioid terminal cells are 8μ or more in diameter, with vacuolar (rarely incrustated) pigment. The cuticle at the apex of the stipe is of repent hyphae, or bears scattered clusters of caulocystidia. Clamps may be present or absent. Most of the species have small to medium-sized, rather thin-fleshed carpophores, but there are some exceptions. A fleshy stature, or frequent clamp connections on the pileal cuticular hyphae, or both, relates some species of *Leptonia* (e.g., *L. jubata*, *L. dichroa*, *L. griseo-cyanea*, and *L. chalybaea*) to *Entoloma*. However,

the species of *Entoloma* differ in their smooth, glabrous pilei, the structure of their pileal cuticle, and the nature of their pilocystidia.

In *Leptonia*, urea concentration varies from 0 to +3. Of 86 collections examined, 78 had little or no urea (0 to +1). Eight collections, including 3 each of *L. dichroa* and *L. jubata*, showed a concentration of +2 to +3. All eight belong to sections of *Leptonia* that do not have a tomentulose pileal disc, thus there seems to be a correlation between urea concentration and the type of pileal surface, which may have significance at the sectional level.

One group of Leptonias, referred to previously as the *L. sericella* species complex, seems different enough from the remainder of the genus to warrant special attention. This group of species has been placed in a new genus, *Alboleptonia* (Largent and Benedict, 1970). This group is characterized by the entirely white to pale cinereous carpophore, the silky to appressed-fibrillose or minutely squamulose pileal surface consisting of an entangled trichodermium, the presence of a fugacious superficial veil, low urea concentration (0 to +1), and a set of unique Ehrlich-positive compounds. The few white Leptonias not included in *Alboleptonia* (*Leptonia albinella* and related species) have the pileal cuticle hymeniform, or consisting of a palisade trichodermium. Pallid or nearly white species of *Entoloma* differ in their glabrous, dry to viscid pilei, larger stature, abundant clamp connections, and dissimilar Ehrlich-positive compounds.

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SUMMARY

As suggested by Tyler, et al. (1965), a study of the urea concentration in species of *Rhodophyllus* proved to be a useful chemotaxonomic aid in classifying these fungi. On the basis primarily of urea concentration, type of pileal surface, and structure of the pileal cuticle, plus the occasional use of other structural or macroscopic features, five genera can be delimited, as follows:

Claudopus. Stipe lacking, or present and lateral to eccentric; place of growth, on the underside of logs or on shelving clumps of moss.

Entoloma. Clamps long, thin, relatively abundant; pileal surface glabrous or frosted; pileal cuticle repent, or an interwoven trichodermium; pilocystidia less than 8μ broad; urea concentration low.

Nolanea. Clamps absent or rare; pileal surface glabrous, dry or lubri-

cous; pileal cuticle of repent hyphae; urea concentration high.

Pouzaromyces. Clamps absent or rare; pileal surface minutely tomentose to densely scaly; surface of stipe pruinose to densely scaly; pileal cuticle of septate, entangled hyphae with dark pigment; cheilocystidia present; apex of stipe with a distinct cuticle; urea concentration not determined.

Leptonia. Clamps usually absent, if present, thick and rather numerous; pileal surface appressed-fibrillose, tomentulose, punctate, or squamulose, at least on the disc; pileal cuticle hymeniform, a palisade trichodermium, an entangled trichodermium, or an intewoven layer of submoniliform hyphae; pilocystidia broad; urea concentration low to medium.

In addition to the above five genera, a distinctive group of species, the *Leptonia sericella* species complex (now *Alboleptonia* Largent & Benedict, 1970), can be characterized as follows: entire corpophore white to pale cinereous; clamps rarely present; pileal surface silky to minutely squamulose; pileal cuticle an entangled trichodermium with a fugacious superficial veil; several unique Ehrlich-positive compounds present.

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A NATURALIZED CORTADERIA (GRAMINEAE) IN CALIFORNIA

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Munz (1968) recorded *Cortaderia selloana* (Schult.) Asch. & Graebn. as naturalized in San Francisco and the North Coast Ranges. He further referred to a heavy infestation of this species at Big Lagoon, Humboldt Co. From the Big Lagoon populations D. W. Cooper of Eureka sent me specimens, transplants, and seed from which plants were raised. All have proved identical with plants widely naturalized in northern North